

Subtropical sacoglossans in Okinawa—at “special risk” or “predictably rare”?

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On low intertidal and shallow subtidal shores on the west coast of Okinawa, Japan, we investigated the trophic associations of sacoglossan opisthobranchs associated with Bryopsidalean green algae. During 11 short research visits (55 days total) from 2002 to 2008, we recorded almost 500 specimens of 11 species. These sacoglossans include a new record for Japan (*Caliphylla* A. Costa, 1867), a recent record for Japan (*Placida daguilarensis* Jensen, 1990), two undescribed species (*Placida* Trinchese, 1876 and *Elysia* Risso, 1818), one unnamed (but well-described) species (*Placida* sp. *sensu* Baba 1986), and six other Indo-Pacific species. Not only did we record more sacoglossan species but also we found higher slug abundances than other colleagues in Okinawa or the Indo-Pacific region. Quantitative population attributes and feeding preferences are described for these sacoglossans. In contrast to temperate geographic regions, several of these Japanese sacoglossans specialized on a single algal genus rather than two or more genera in different families. This specificity is consistent with narrower host-plant associations in high-diversity communities; yet monophagy has not yet been demonstrated in this guild of Okinawan sacoglossans. Given the broad geographic ranges, restricted host ranges, often predictable populations, and high frequency of life cycles with planktotrophic larvae, western Pacific subtropical sacoglossans should be considered “predictably rare” (*sensu* Rabinowitz 1981) rather than at “special risk” (*sensu* Clark 1994).

Key words: Sacoglossa, Japan, herbivory, *Codium*, *Bryopsis*

Western Pacific tropical and subtropical shores are characterized by the high species richness of the marine fauna and flora. The taxonomic and ecological diversity may reflect the cumulative effects of at least two biogeographic patterns: (1) Western Pacific shores are more diverse than eastern Pacific shores of comparable latitude, (2) Species richness increases along a latitudinal gradient from polar to tropical areas. Geographic patterns of marine specialist herbivores, particularly the sacoglossan opisthobranchs, parallel these general ecological patterns. Many areas of the subtropical and tropical Pacific and Indo-Pacific are considered to be biodiversity “hotspots” for many taxonomic groups from coastal and pelagic fishes to seaweeds and invertebrates. Recent studies by Gosliner (1992), Ono (1999, 2004), Carlson and Hoff (2003), Jensen (2007), and Gosliner *et al.* (2008) exemplify this pattern.

In contrast to these natural patterns, anthropogenic activities may reduce biodiversity by habitat destruction, introduced species' effects, eutrophication, and other forms of pollution. Because anthropogenic activities are disproportionately acute on tropical and subtropical shores affected by tourism and/or harvesting, coastal communities are often

degraded or biologically compromised. Clark and DeFreese (1987) and Clark (1994) discussed the problems as they relate to sacoglossan occurrence, with Clark suggesting that the specialists are at “special risk” of extinction because of their host specificity and high frequency of direct development. He presented data on the decline of Key West populations of sacoglossans. In most other cases, the importance of natural vs. anthropogenic effects has often been asserted, rather than quantitatively demonstrated, for tropical and subtropical shores.

As part of a large-scale project on Japanese sacoglossan assemblages (Hirano *et al.* 2003, 2005, 2006a, 2006b, 2006c, 2007a, 2007b, 2007c, 2008, Shimadu 2004, Trowbridge *et al.* 2005, 2006, 2007, 2008a, 2008b, 2009a, 2009b, Shimadu *et al.* 2006, Kumagai 2009), we quantified spatial, temporal, and taxonomic patterns of sacoglossan opisthobranchs and their macroalgal hosts on the subtropical shores of western Okinawa. Anthropogenic effects on Okinawa shores include habitat destruction, nutrient loading, sedimentation, heavy metal accumulation, human trampling, over-harvesting (fishes, invertebrates, and seaweeds), coral reef bleaching, etc. Although we did not document anthropogenic effects

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during this study, one of our sites was severely disturbed by a shoreline modification project that replaced the natural shoreline with coral blocks and added a breakwater during our study (Fig. 1). Our other sites were heavily used recreational sites for scuba divers, swimmers, and collectors. Despite these anthropogenic effects, we recorded impressively high species richness of sacoglossan gastropods.

The regional diversity of sacoglossans in Fiji, Guam, Thailand, Hong Kong, and Japan is well known to be extremely high (Carlson and Hoff 2003, Jensen 2007, Trowbridge *et al.* 2007, unpubl. ms, Gosliner *et al.* 2008). However, the local alpha- and beta-diversity (*e.g.*, within individual sites or among closely adjacent sites) and the population abundance of sacoglossans in the Ryukyu Islands of Japan, particularly on the main island of Okinawa, have traditionally been considered low (see Bolland's Okinawa Slug Site at <http://www.rfbolland.com/okislugs/>). We quantitatively demonstrate that

local-scale assemblages of sacoglossans are not only diverse but also reliably found. Okinawan shores are clearly a "hot-spot" of sacoglossan biodiversity despite intense anthropogenic effects.

In this account, we focus on sacoglossan gastropods associated with hosts belonging to the green algal order Bryopsidales (taxonomy based on Guiry and Guiry 2009). Specifically, we investigated slugs on *Codium* (F. Codiaceae), *Bryopsis* (F. Bryopsidaceae), and *Derbesia* (F. Derbesiaceae). On NW and NE Atlantic shores, NE Pacific shores, S Pacific shores, and perhaps elsewhere, sacoglossans feed on 2-3 of these genera and, thus, are feeding on species in 2-3 algal families. Notable examples include *Placida dendritica* (Alder and Hancock, 1843), *Elysia viridis* (Montagu, 1804), *Elysia hedgpethi* Er. Marcus, 1961, and *Placida* sp. (*sensu* Baba 1986). However, in Florida *Placida kingstoni* Thompson, 1977 appears to feed on *Bryopsis* but not *Codium*. Furthermore, *Elysia trisinuata* Baba, 1949 in Hong Kong and Japan apparently feeds exclusively on *Codium* (Jensen 2003, Trowbridge *et al.* 2008a). The ecological and conservation implications of diet breadth are far-reaching. In particular, species with a greater suite of alternate hosts would be more buffered from disturbance or habitat loss than monophagous species. Understanding the feeding specificity and population abundances of specialist gastropods will enable us to determine whether they are "special risk" as suggested by Clark (1994) or merely "predictably rare" (*sensu* Rabinowitz 1981).

MATERIALS AND METHODS

Study region

The Ryukyu Archipelago extends from Kyushu to Taiwan and has several major island groups, each composed of numerous smaller islands. In the Ryukyu Shoto (the southern half of the islands), the Okinawa Islands are considered the central group or Ryukyu proper (Wikipedia 2009). The largest island in this group, Okinawa (also known as the Okinawan mainland or Okinawajima), is 1201 km² in area. We examined sacoglossans on the west coast of Okinawa, facing the East China Sea.

The climate of Okinawa is subtropical with mean air temperature of 23 °C, mean seawater temperature of 25 °C, and annual rainfall of >2 m. The tidal range is ca. 2.2 m on the western shore of Okinawa. Spring low tides expose coral reef platforms with diverse and abundant siphonous green algae (*e.g.*, *Codium*, *Bryopsis*, *Caulerpa*, *Halimeda*, and allies). Algal populations also occur in shallow subtidal habitats (*e.g.*, shallow fringing lagoons, reef crest, and reef slope). The siphonous green algal communities are flourishing despite intense trampling, marine species' collection, nutrient enhancement, shoreline modification, and water sports.



Figure 1. Anthropogenic disturbance at Sobe, Okinawa during our study: coastal modification to protect shore from storm waves. A, Replacement of vegetation and shoreline with coral blocks and sand; downward movement of coarse sand smothered intertidal and subtidal organisms. B, Addition of breakwater that changed the hydrology of site, including the shifting of the seagrass meadows and other marine macrophytes.

Surveys

We visited the main island of Okinawa 11 times from July 2002 to December 2008 (Table 1). We surveyed the shore for 4–6 days per trip over a 6-y period including a variety of seasons. We concentrated our surveys at three primary sites between Naha and Zampa-misaki on the west coast. From north to south, these sites were Zampa (26°26'15"N, 127°42'48"E), Sobe (26°23'12"N, 127°43'24"E), and Sunabe (26°19'51"N, 127°44'36"E). We surveyed the low intertidal and shallow subtidal area of the shore where seaweeds grow profusely. This habitat has been understudied, as the majority of searches for opisthobranchs have involved scuba diving. We snorkeled over the intertidal zone during mid-tide, and the shallow subtidal area (<0.5 m) during low tide. During each survey, we collected Bryopsidalean green algae (*Codium*, *Bryopsis*, and *Derbesia*) and subsequently investigated these in shallow trays on the shore. Slugs used in the feeding experiments were collected from the trays (for small species) or directly from submerged algal hosts in the field (for larger species). Slug abundances were determined on an algal biomass basis (#/kg wet weight of algae).

Seaweeds of the NW Pacific are challengingly diverse. For algal identifications, we relied on Yoshida (1998) and Yoshida *et al.* (2005) as well as phylogenetic expertise of colleagues in Okinawa (Prof. S. Kamura), mainland Japan (Prof. T. Kobara), and Taiwan (Dr. Jui-Sheng Chang). *Codium repens*—long reported for Okinawa—is an Atlantic species; Pacific specimens reported by that name belong to a morphologically complex series of species recently reviewed by Chang *et al.* (2002); Dr. Chang kindly identified our specimens as *Codium geppiorum*.

Species documentation

For all the species, we took high-resolution digital images to document coloration of living individuals. Voucher specimens were measured to the nearest millimeter, relaxed in

3.8% MgCl₂, and then preserved in 5% buffered formalin; vouchers were deposited in the extensive Hirano opisthobranch collection at Chiba University. Our initial identifications were based on external morphological characters; subsequent dissections of radulae and reproductive systems were made to confirm species identifications relative to type descriptions.

Feeding experiments

Sacoglossans were used for two types of feeding experiments when available. In 16 pairwise-choice experiments (Tables 2–4), individual slugs were placed in separate containers with pairwise choices of different green algae known to be host plants of sacoglossans. Containers (ca. 150 ml) were filled with freshly collected seawater and held at room temperature. Periodic observations were made over a 1 or 2-d period, monitoring the location of each individual relative to the algal choices. At the end of each experiment, grazing damage was noted. Such experiments are crucial to demonstrate what a sacoglossan prefers to consume, but not whether it could or would consume the less-attractive choice. We also conducted six no-choice experiments to see if specimens would feed on other potential hosts; feeding was determined by recording any visible grazing damage to the algae and/or freshly acquired chloroplasts in the sacoglossan diverticulae (readily visible in most sacoglossans).

Because the literature presents confusing and indirect information about one sacoglossan's algal hosts, we conducted eight feeding trials with the undescribed species of *Placida* Trinchese, 1876. Despite superficial similarities with *Placida dendritica*, *Placida daguilarensis* Jensen, 1990, and *Placida* sp. (*sensu* Baba 1986), there are numerous external and internal differences between the undescribed Okinawan *Placida* sp. and these other three, well-described *Placida* species. Published records or photographs of the sacoglossan on *Caulerpa sertularioides*, *Caulerpa lentillifera*, and congeners (e.g., Ichikawa 1993, Ono 1999) prompted us to investigate whether *Placida* sp. could or would consume *Caulerpa*. We also evaluated whether this species would eat *Bryopsis*, *Derbesia*, *Valonia*, or the septate *Rhizoclonium* (Table 2).

With *Elysia trisimata*, we tested whether the species exhibited a preference between coexisting *Codium* species and whether the slug would eat *Bryopsis* in a no-choice situation (Table 3). With *Stiliger* spp., we conducted four pairwise-choice and two no-choice experiments to evaluate sacoglossans' preference and capacity to feed on the sympatric *Codium geppiorum*, *C. arabicum*, and *Bryopsis harveyana*. For *Elysia ornata* (Swainson, 1840), we conducted four experiments to test whether the species would consume the non-host *Derbesia* or *Codium* as well as whether the species would consume sympatric *Bryopsis* spp. Finally, for *Placida crenoniana* (Trinchese, 1893), we were constrained by small

Table 1. List of survey dates for sacoglossan surveys in Okinawa, Japan.

Year	Month	Number of actual survey days *
2002	July 7–12	6
2003	November 4–10	6
2004	March 8–11; July 1–6; November 22–25	4; 4; 4
2005	March 10–15; November 27–December 2	6; 6
2006	March 28–April 1; December 18–24	5; 5
2007	March 4–8	4
2008	December 10–16	5

* Inclement weather or sea conditions prevented us from surveying every day.

Table 2. Feeding preference experiments conducted with *Placida* sp. (undescribed) in Okinawa between 2002 and 2006.

Date	Experimental design	Result
July 2002	- pairwise choice of <i>Codium geppiorum</i> (host) and <i>Caulerpa sertularioides</i> <i>N</i> = 22 slugs	- slugs very strongly preferred <i>Codium</i>; none fed on <i>Caulerpa</i> although a few specimens explored the alga - overall 65% on <i>Codium</i> and 7% on <i>Caulerpa</i> 86% on <i>Codium</i> and 0% on <i>Caulerpa</i> at 24 h
July 2002	- pairwise choice of <i>Bryopsis harveyana</i> and <i>Caulerpa sertularioides</i> <i>N</i> = 21 slugs	- slugs preferred <i>Bryopsis</i> to <i>Caulerpa</i> but did not like either choice (few explored either alga); no algae eaten - overall 28% on <i>Bryopsis</i> and 12% on <i>Caulerpa</i>
November 2003	- pairwise choice of <i>Codium geppiorum</i> (host) and <i>C. arabicum</i> <i>N</i> = 4 slugs	- slugs strongly preferred <i>Codium geppiorum</i>; only 1 even explored <i>C. arabicum</i> - overall 53% on <i>C. geppiorum</i> and 9% on <i>C. arabicum</i>
November 2003	- pairwise choice of <i>Bryopsis harveyana</i> and <i>Derbesia</i> sp. <i>N</i> = 2 slugs	- slugs preferred associating with <i>Derbesia</i>; no evidence of feeding on either alga - overall 64% on <i>Derbesia</i> and 9% on <i>Bryopsis</i>
March 2004	- pairwise choice of <i>Codium geppiorum</i> (host) and <i>Valonia aegagropila</i> <i>N</i> = 45 slugs	- slugs strongly preferred <i>Codium</i>; did not eat <i>Valonia</i> - overall 56% on <i>Codium</i> and 0% on <i>Valonia</i>
November 2005	- no-choice design of <i>Bryopsis harveyana</i> <i>N</i> = 1 slug	- slug on alga but did not or could not feed - overall 92% on <i>Bryopsis</i>
March 2006	- pairwise choice of <i>Codium geppiorum</i> (host) and <i>Bryopsis harveyana</i> <i>N</i> = 18 slugs	- slugs strongly preferred <i>Codium</i>; did not eat <i>Bryopsis</i> - overall 54% on <i>Codium</i> and 15% on <i>Bryopsis</i>
March 2006	- pairwise choice of <i>Bryopsis harveyana</i> and <i>Rhizoclonium grande</i> <i>N</i> = 18 slugs	- slugs preferred to explore <i>Bryopsis</i> to <i>Rhizoclonium</i> but did not like either choice; no algae eaten - overall 18% on <i>Bryopsis</i> and 8% on <i>Rhizoclonium</i>

sample sizes (*N* = 3) so conducted just two preference experiments: between *Codium* spp. and between *Derbesia* and *Bryopsis*.

RESULTS

Environmental conditions

In July, the mean seawater temperature during each survey averaged 30–32 °C. In November and December, the mean seawater temperature ranged from 20.5 to 25.6 °C. Finally, during March surveys, mean seawater temperature varied from 20.0 to 22.0 °C. Thus, in nearshore habitats, the annual temperature range was at least 12 °C. This pronounced seasonality was associated with the strongly seasonal changes in macroalgal and sacoglossan assemblages.

Sacoglossans

The species recorded on Bryopsidalean green algae during this 6-y survey included seven cerata-bearing species and four elysids (Figs. 2–3). These species were (1) an undescribed *Placida* Trinchese, 1876, (2) the well-described *Placida* sp. (*sensu* Baba 1986), (3) *Placida cremoniana* (Trinchese, 1893),

(4) *Placida daguilaensis* Jensen, 1990, (5) *Stiliger ornatus* Ehrenberg, 1828, (6) *Stiliger aureomarginatus* Jensen, 1993, (7) a species of *Caliphylia* A. Costa, 1867, (8) *Elysia trisinuata* Baba, 1949, (9) *Elysia ornata* (*sensu lato*) (Swainson, 1840), (10) *Elysia rufescens* (Pease, 1871), and (11) an undescribed *Elysia* Risso, 1818.

Codium associations

Five sacoglossan species that feed on and associate with the perennial green algal host *Codium* were present during our surveys (Fig. 2). In particular, the small (≤ 5 mm), undescribed *Placida* sp. (Fig. 2A) was reliably found on the green macroalga *Codium geppiorum* and the sympatric encrusting congener *Codium arabicum* at Sobe and, to a lesser degree, at the other two sites. The species was more common on thalli on the seaward side of the reef crest than on the landward side. For example, in March 2005, the species was 2.3 times more common (based on number per kg wet weight of *Codium*) on the seaward than the landward side of the fringing reef. We collected >320 specimens of *Placida* sp. (with comparatively little effort) between 2002 and 2008. Peak abundances were recorded in March surveys (Figs. 4–5) when we found 57 to 87 specimens per trip. The species was significantly more abundant in March than other survey times

Table 3. Feeding preference experiments conducted with three other sacoglossan species from *Codium* spp.

Sacoglossan species	Date	Experimental design	Results
<i>Elysia trisinuata</i>	November 2003	● pairwise choice of <i>Codium geppiorum</i> (host) and <i>C. arabicum</i> ● <i>N</i> = 6 slugs	● strongly preferred <i>Codium geppiorum</i>; only 1 slug even briefly explored <i>C. arabicum</i> ● overall 77% on <i>C. geppiorum</i> and 7% on <i>C. arabicum</i>
<i>Elysia trisinuata</i>	November 2005	● no-choice design of <i>Bryopsis harveyana</i> ● <i>N</i> = 8 slugs	● 4 slugs tried to feed but no cell sap removed; 2 slugs laid eggs on alga; other 2 slugs avoided alga
<i>Stiliger aureo-marginatus</i>	March 2005	● no-choice design of <i>Bryopsis harveyana</i> ● <i>N</i> = 15 slugs	● only 1 slug even explored <i>Bryopsis</i>; no feeding ● overall 3% on <i>Bryopsis</i>
<i>Stiliger aureo-marginatus</i>	March 2005	● pairwise choice of <i>Codium arabicum</i> and <i>Bryopsis harveyana</i> ● <i>N</i> = 15 slugs	● slugs strongly preferred <i>Codium</i>; only 2 slugs even explored <i>Bryopsis</i> but did not feed on it ● overall 53% on <i>Codium</i> and 8% on <i>Bryopsis</i>
<i>Stiliger aureo-marginatus</i>	March 2005	● pairwise choice of <i>Codium geppiorum</i> (host of 9) and <i>C. arabicum</i> (host of 2) ● <i>N</i> = 11 slugs	● fed on both <i>Codium</i> species irrespective of host source ● overall 41% on <i>C. geppiorum</i> and 43% on <i>C. arabicum</i>
<i>Stiliger ornatus</i>	November 2003	● pairwise choice of <i>Codium geppiorum</i> (host) and <i>C. arabicum</i> ● <i>N</i> = 2 slugs	● strongly preferred <i>Codium geppiorum</i>; only 1 slug even briefly explored <i>C. arabicum</i> ● overall 45% on <i>C. geppiorum</i> and 5% on <i>C. arabicum</i>
<i>Stiliger ornatus</i>	March 2005	● pairwise choice of <i>Codium geppiorum</i> (host) and <i>C. arabicum</i> ● <i>N</i> = 1 slug	● slug preferred <i>Codium geppiorum</i> ● overall 55% on <i>C. geppiorum</i> and 9% on <i>C. arabicum</i>
<i>Stiliger ornatus</i>	March 2006	● no-choice design of <i>Bryopsis harveyana</i> ● <i>N</i> = 12 slugs	● only 2 slugs even explored <i>Bryopsis</i>; they did not feed on the alga ● overall 8% on <i>Bryopsis</i>

(Kruskal-Wallis, $H = 10.7$, $P = 0.005$). The mean March abundance was ca. 40 individuals per kg *Codium* (wet weight) (Fig. 5A).

Based on pairwise-choice experiments (Table 2), *Placida* sp. from *Codium geppiorum* (1) strongly preferred *C. geppiorum* to *Caulerpa sertularioides* and *Valonia aegagropila*, (2) preferred *Codium* to *Bryopsis harveyana*, (3) preferred *C. geppiorum* to sympatric congener *C. arabicum*, and (4) preferred *Derbesia* sp. to *B. harveyana*. These results and the field associations indicate that *Codium* spp. are primary hosts to *Placida* sp. Although *Placida* sp. did explore *Derbesia* and *Bryopsis* in experiments, we observed no actual evidence of feeding and documented no field associations; the algae do not appear to be hosts. Furthermore, based on the lack of these slugs on *Caulerpa* and *Valonia*, these algae are clearly not hosts; there was no direct or indirect evidence of *Placida* ever feeding on the delicate *Caulerpa sertularioides* or occurring on the alga in the field during our study.

Coexisting sacoglossans on *Codium* spp. included *Placida* sp. (*sensu* Baba 1986) (Fig. 2B), *Stiliger aureomarginatus* (Figs. 2D-E), *Stiliger ornatus* (Fig. 2F), and *Elysia trisinuata* (Fig. 2G). The well-described but unnamed *Placida* sp. was observed in 3 of the 11 surveys (March 2005, 2006, and 2007); two authors (Shimadu and Kumagai) conducted their thesis

research on this species in eastern Honshu, thus aiding in our recognition of the typically more northern species.

The small (≤ 5 mm), brightly colored sacoglossan *Stiliger aureomarginatus* was seen on *Codium geppiorum* primarily in March surveys (Figs. 4A, 5C); this report constitutes the first formal record of this species for Okinawa. Peak densities were recorded on 7 March 2007 with 23 individuals per 545 g *C. geppiorum*. This sacoglossan species is easily confused with the small (≤ 5 mm) *Stiliger ornatus*, particularly with juvenile slugs; the key distinguishing feature is the color pattern on the cerata: dark cerata with orange tips (Figs. 2D-E). In contrast, *S. ornatus* specimens have cerata with a yellow proximal area, then a dark blue to black encircling band, followed by a yellow band, and then a dark tip (Fig. 2F). *Stiliger ornatus* was most abundant in July (Figs. 4A, 5D) although not significantly so (Kruskal-Wallis test, $H = 2.0$, $P = 0.360$) due to the large variation among July collections; for both species, grazing damage to *Codium geppiorum* was highly visible. In feeding experiments (Table 3), the two species strongly preferred *Codium* to *Bryopsis* and would not consume the latter in no-choice trials. *Stiliger aureomarginatus* exhibited no preference between congeneric *Codium* spp. Although *S. ornatus* preferred its host (*C. geppiorum*) to the coexisting *C. arabicum*, this may reflect the small sample size ($N = 2$).

Table 4. Feeding preference experiments conducted with *Elysia ornata* from *Bryopsis* and *Placida cremoniana* from *Derbesia* in Okinawa between 2002 and 2008.

Sacoglossan species	Date	Experimental design	Results
<i>Elysia ornata</i>	November 2003	- pairwise choice of <i>Bryopsis harveyana</i> (host) and <i>Derbesia</i> sp. $N = 7$ slugs	- slugs strongly preferred <i>Bryopsis</i>; few slugs explored <i>Derbesia</i> and covered it with mucus; no damage to <i>Derbesia</i> - overall 81% on <i>Bryopsis</i> and 5% on <i>Derbesia</i>
<i>Elysia ornata</i>	November 2003	- no-choice design of either <i>Bryopsis harveyana</i> (host) or <i>Derbesia</i> sp. $N = 8$ slugs	- all <i>Bryopsis</i> was consumed - no <i>Derbesia</i> was consumed; slugs laid eggs on <i>Derbesia</i> or sat on it but did not eat it - overall 48% on <i>Bryopsis</i> and 31% on <i>Derbesia</i>
<i>Elysia ornata</i>	March 2005	- no-choice design of <i>Codium arabicum</i> $N = 6$ slugs	3 slugs briefly explored alga but did not feed
<i>Elysia ornata</i>	March 2005	- pairwise choice of <i>Bryopsis harveyana</i> and <i>Bryopsis</i> sp. (host unknown) $N = 4$ slugs	- slugs explored and consumed both <i>Bryopsis</i> species - overall 18% on <i>Bryopsis</i>
<i>Placida cremoniana</i>	November 2003	- pairwise choice of <i>Codium geppiorum</i> and <i>C. arabicum</i> $N = 3$ slugs	- strongly disliked both <i>Codium</i> spp. - overall 10% on <i>C. geppiorum</i> and 0% on <i>C. arabicum</i>
<i>Placida cremoniana</i>	November 2003	- pairwise choice of <i>Bryopsis harveyana</i> and <i>Derbesia</i> sp. (host) $N = 3$ slugs	1 small juvenile fed on <i>Bryopsis</i> and grew; other 2 larger slugs fed only on <i>Derbesia</i> - overall 56% on <i>Derbesia</i> and 17% on <i>Bryopsis</i>

Elysia trisinuata (Fig. 2G) was found in March, July, and November/December and was comparatively large (20–30 mm long). There was no significant seasonality in *Elysia* abundance ($H = 4.7$, $P = 0.093$) although March collections tended to be the smallest. In feeding experiments, *E. trisinuata* from *Codium geppiorum* preferred its host to the coexisting but less common *C. arabicum*; the reciprocal experiment was not feasible but a few slugs were associated with *C. arabicum* on the shore. In a no-choice situation, this species would not eat *Bryopsis*.

***Bryopsis* associations**

The delicately branching *Bryopsis* and *Derbesia* species were seasonal or ephemeral in Okinawa. Six species of sacoglossans were associated with the abundant *Bryopsis harveyana* and less common congeners: *Elysia ornata* (*sensu lato*), *Elysia rufescens*, an undescribed *Elysia* sp., *Placida daguilarensis*, *Placida* sp. (*sensu* Baba 1986), and *Caliphylla* sp.

Elysia ornata (Fig. 3A–C) was reliably associated with *Bryopsis*, particularly in the November/December and March surveys when seawater temperatures were low and *Bryopsis* was abundant in the shallow subtidal areas, particularly near the reef edge. The sacoglossan species was large with specimens up to 52 mm long. We noted that specimens varied from pale body coloration to bright green, depending on the time since

feeding on *Bryopsis*. More importantly, however, the rhinophore color varied from bright orange or red (Figs. 3A–B) to green with only a faint tinge of red (Fig. 3C); the rhinophores lacked the black markings observed in the undescribed *Elysia* sp. (Fig. 3F). Black markings on the body surface of *E. ornata*, including inside and outside parapodia, varied from large (Fig. 3A) to small (Figs. 3B–C). In a pairwise-choice experiment (Table 4), *E. ornata* strongly preferred its host *Bryopsis harveyana* to the morphologically similar *Derbesia* sp. In no-choice experiments, *E. ornata* specimens consumed all the *Bryopsis* but none of the *Derbesia*; they crawled all over *Derbesia* and spawned on it, but did not feed on it.

Elysia rufescens also was frequent on *Bryopsis* and reached large sizes (42 mm). This species was less common than the large congener *Elysia ornata* (Fig. 4B). No feeding experiments were conducted with *E. rufescens* during this study; however, the species was not found in association with any algal host other than *Bryopsis* (we searched ca. 15 genera of green algae and many genera of red algal hosts during the large-scale study).

In November 2004, an undescribed *Elysia* sp. (Fig. 3F) was found crawling on the benthos, not in association with any alga, but near *Elysia ornata* specimens which were in a habitat where small *Bryopsis* thalli grew in rock crevices and holes. However, the 50-mm sacoglossan readily consumed *Bryopsis* in the laboratory. This species, recognized by many colleagues as undescribed, may have been recorded on other

dates. The primary distinguishing features of the undescribed *Elysia* sp. are the three pairs of parapodial thickenings that are tinged orange and the rhinophores with black markings. In *E. ornata*, the orange and white parapodial lines are typically continuous and the rhinophore coloration is distinct (Figs. 3A-C). The other had black dots and larger, more diffuse white markings inside and outside the parapodia. Detailed comparisons of the internal anatomy of the undescribed species and *E. ornata* (*sensu lato*) have not yet been made by our research group (or others).

A small *Placida* species (to 8 mm long) was recorded from *Bryopsis* (Fig. 3E). At first, the animal was lumped with *Placida* sp. (*sensu* Baba 1986). After investigating the internal anatomy of the former, the species was positively identified as *Placida daguilaensis* Jensen, 1990 and reported as a new record for Japan (see Hirano *et al.* 2006b, 2006c). Because of our photographic technique and detailed notes, we were able to retroactively distinguish the species in our early records. The differences between *P. daguilaensis* (Fig. 3E) and the other *Placida* species include external morphology such as the shape of the cerata and the pericardium (Figs. 2A-C, 3E), internal morphology (reproductive system), branching patterns of digestive diverticulae (based on squash mounts of cerata), and algal diet. *Placida daguilaensis*, originally described from Hong Kong, was not particularly common during our Okinawan surveys but we found many additional specimens in Honshu.

Placida sp. (*sensu* Baba 1986) in Okinawa was primarily found on *Codium*. However, on 7 March 2007, we found one individual (8 mm) on *Bryopsis harveyana* (Fig. 2C). Consistent with our work on Honshu, specimens on *Bryopsis* had longer cerata and sparser digestive diverticulae on the rhinophores, head, and dorsum than conspecifics on *Codium* (Fig. 2B).

Finally, another new species record for Japan is the cerata-bearing *Caliphylla* sp. To date, we have found two specimens of *Caliphylla* (Fig. 3G) in December 2006 at Sobe (12 and 17 mm). In December 2008, we did not find the species despite intensive searching; however, *Bryopsis* populations were not as lush as normal due to prolonged persistence of warm-water in autumn. After investigating the internal anatomy of this species, examining the literature, and reviewing our previous photographic and specimen-based records, we also found *Caliphylla* sp. from Sagami Bay on Honshu in November 2002.

***Derbesia* associations**

The rather flamboyant *Placida cremoniana* (Fig. 3H) was found in association with the green alga *Derbesia* sp. that was entangled around other seaweeds (e.g., among the calcareous fronds of branching coralline red algae). This was the first apparent record of the sporophyte stage of *Derbesia*

in Okinawa. In a pairwise-choice trial (Table 4), *P. cremoniana* preferred its algal host (*Derbesia*) to *Bryopsis* but we need more Okinawan specimens to test this more rigorously as one small juvenile fed on *Bryopsis* and grew.

DISCUSSION

Species richness

Our surveys have yielded several important results about the species richness, trophic associations, and population abundances of sacoglossan opisthobranchs. From 2002 to 2008, we recorded 11 species of sacoglossans associated with Bryopsidalean green algae in Okinawa. This value was slightly higher than the 9 species recorded by Gosliner *et al.* (2008) for the Indo-Pacific region. The only Indo-Pacific species in this feeding guild that we did not record was *Elysia punctata* Kelaart, 1858 associated with *Bryopsis*; based on the species range reported by Gosliner *et al.* (2008), *E. punctata* has not yet been recorded in Japan and environs. We consider that our undescribed *Elysia* sp. that feeds on *Bryopsis* probably corresponds to *Elysia* sp. 11 of the *Sea Slug Forum* and *Elysia* sp. 3 of Ono (2004). Whether our species also corresponds to *Elysia* sp. 2 of Gosliner *et al.* (2008) is unclear as their specimen had a distinctive purple color inside the parapodia that our specimens lacked.

This paper reports the new record of *Caliphylla* in Japan (previously reported by Hirano *et al.* 2007a). The Okinawan record is not the first record of the genus in the western Pacific or Indo-Pacific region; for example, Carlson and Hoff (2003) reported *Caliphylla mediterranea* A. Costa, 1867. However, based on the reproductive system, our species does not appear to be the described *C. mediterranea*. Gosliner *et al.* (2008) also reported an undescribed *Caliphylla* sp. from Hawaii. More detailed ecological, morphological, and molecular investigations need to be conducted before synonymizing the NW Pacific species with the Mediterranean one. Furthermore, the recent reports by Camacho-Garcia *et al.* (2005) and (posthumously) by Jim Lance for the Mexican Pacific (Hertz 2006, Steinberg 2007) and other reports of *Caliphylla* sp. for the tropical NE Pacific merit reexamination of the genus.

Furthermore, the status of *Elysia ornata* as a single species or as an assemblage of cryptic species (including *Elysia marginata* (Pease, 1871) and *Elysia grandifolia* Kelaart, 1858) is unclear (Jensen 1992, 2001, Jensen and Padmakumar 1999, Rudman 1999, Carlson and Hoff 1978, 2003). We therefore refer to this "species" as *E. ornata* (*sensu lato*). Some of this variation is apparent when comparing Figs. 3A-C and numerous photographs in Ono (1999, 2004), Gosliner *et al.* (2008), and *Sea Slug Forum* (<http://seaslugforum.net/>).

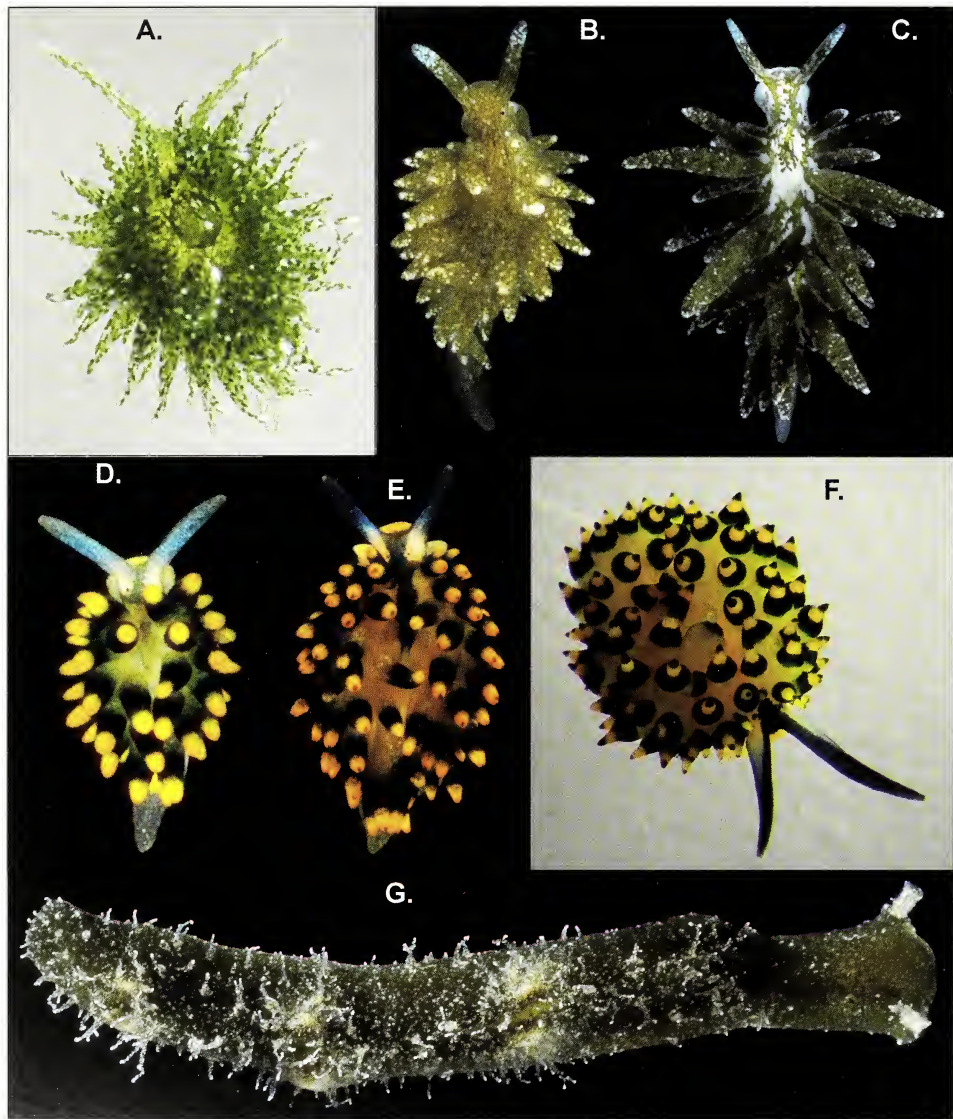




Figure 3. Sacoglossans collected from the green algae *Bryopsis* spp. (mostly *B. harveyana*) (A-G) or *Derbesia* sp. (H) between 2002 and 2008. A-C, Specimens of *Elysia ornata* (*sensu lato*): A (19 mm, 20 December 2006, Sobe); B (35 mm, 5 July 2004, Sobe); and C (40 mm, 10 December 2008, Sobe). D, *Elysia rufescens* (32 mm, 10 December 2008, Sobe). E, *Placida daguilarensis* (8 mm, 2 December 2005, Sobe). F, *Elysia* sp. (50 mm, 22 November 2004, Sobe). G, *Caliphylia* sp. (12 mm, 23 December 2006, Sobe). H, *Placida cremoniana* (8 mm, 7 November 2003, Sobe).

Figure 2. Sacoglossans collected from the green algae *Codium geppiorum* and/or *Codium arabicum* (except for C) during surveys between 2002 and 2008. A, Undescribed *Placida* sp. (4 mm, 10 December 2008, Sobe). B, *Placida* sp. (*sensu* Baba 1986) (4 mm, 28 March 2006, Sobe). C, *Placida* sp. (*sensu* Baba 1986) (8 mm, 7 March 2007, specimen from *Bryopsis harveyana*, Toguchi, Okinawa). D, Juvenile specimen of *Stiliger aureomarginatus* (3 mm, 28 March 2006, Sobe). E, Adult specimen of *S. aureomarginatus* (6 mm, 10 March 2005, Sobe). F, *Stiliger ornatus* (6 mm, 10 December 2008, Sobe). G, *Elysia trisinuata* (29 mm, 29 November 2005, Sobe).

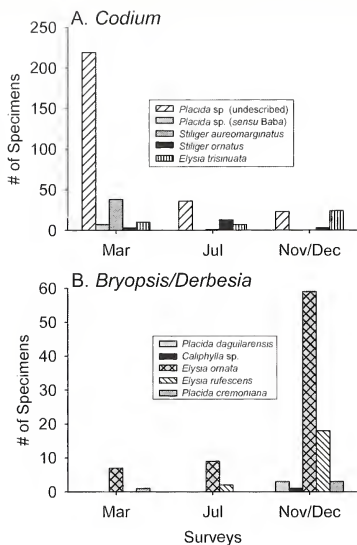


Figure 4. Number of specimens collected in different seasons from 2002 to 2008. A, Sacoglossan species associated with *Codium* spp. B, Sacoglossans associated with *Bryopsis* (first four species) or *Derbesia* (fifth species).

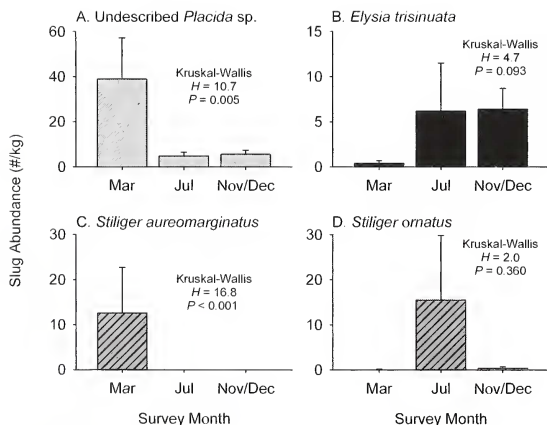


Figure 5. Abundance of three genera of sacoglossans on *Codium* spp. (mostly *C. geppiorum*) on Okinawajima shores, Japan.

Trophic associations

Overview

We recorded field associations and/or laboratory diets of 11 species of Okinawan sacoglossans. Based on our data and published records of other malacologists, we calculated the trophic associations of each species: the frequent hosts as well as the minor associations (Fig. 6). Based on our 6-y study and the literature, ten species (91%) typically or frequently fed on and associated with algal hosts in a single family; one species (9%) fed on hosts in two families. In contrast, on NE Pacific, NW Atlantic, and NE Atlantic shores, sacoglossans typically feed on 2-3 families of Bryopsidalean algae.

If we broadened our focus to include all records of association and/or feeding across each species' geographic range, the pattern differs (Fig. 6). Seven of these sacoglossan species (64%) fed on hosts within the same algal family whereas three species (27%) fed on two families of Bryopsidalean green algae and one species (9%) fed on three families. In ecological and evolutionary terms, how significant are the infrequent or unusual host associations? On one hand, the broader diets do not reflect what the majority of members of a population or species do. On the other hand, the exceptions or the infrequent records may reflect what host-switches starving sacoglossans can and will do when they have depleted ephemeral hosts (e.g., *Bryopsis*). Most of the "infrequent" records are due to the occasional association of *Bryopsis*-feeding sacoglossans with *Derbesia* or *Pedobesia* (F. Derbesiaceae) or of *Derbesia*-feeding sacoglossans with *Bryopsis* or *Pseudoderbesia* (F. Bryopsidaceae).

Details

Many of the associations were expected (based on the published literature and from internet reports). However, we substantially improved the understanding of several species. For example, the host association of *Placidia cremoniana*, a species described in 1893, has long eluded malacologists: we recorded herein the Okinawan specimens associated with and fed on *Derbesia*. Additional work in Sagami Bay, Honshu (Hirano *et al.* 2007c) confirmed that *P. cremoniana* feeds on *Derbesia* as well as on a related alga *Pedobesia ryukyensis* (F. Derbesiaceae). Both genera have the sporophyte (diploid) stage as dominant in the life cycle. In contrast, *P. cremoniana* would not readily consume *Bryopsis* or *Pseudoderbesia* which have the gametophyte (haploid) stage dominant. Although

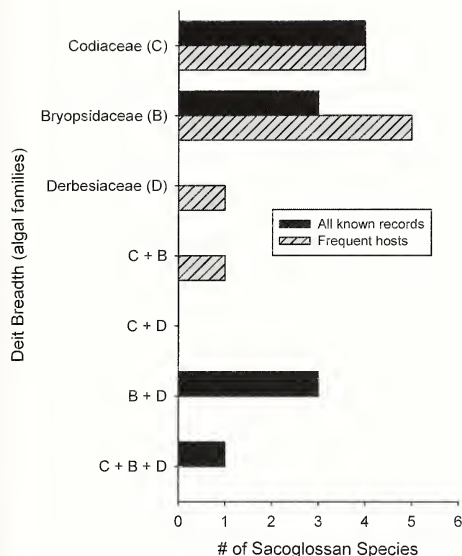


Figure 6. Diet breadth of sacoglossans associated with Bryopsidalean algae. Bars indicate the number of Okinawan species to feed within a single Bryopsidalean family or between two or more families. Data based on this study and the literature.

some previous studies have suggested that Bryopsidalean sporophytes have the polysaccharide mannan as a primary constituent of cell walls and gametophytes have the polysaccharide xylan in walls, phycologists emphasize that these patterns are based on only a few algal species and are not absolute: life-history stage and wall chemistry are not necessarily linked. Thus, we do not extrapolate about cell wall constituents of Okinawan algae vs. sacoglossan tooth shape. However, we do note that *P. cremoniana* has strikingly small teeth compared to other sacoglossans that feed on Bryopsidalean algae.

Malacologists have long recognized that *Elysia ornata* (*sensu lato*) feeds on *Bryopsis*. However, Hirano *et al.* (2007c) recently reported that in Japan the species also feeds on *Pseudoderbesia* (F. Bryopsidaceae) and *Pedobesia* (F. Derbesiaceae). Thus, *E. ornata* is not monophagous: it feeds on many species of *Bryopsis* and on three genera in two algal families. This result has considerable applied significance as *E. ornata* and the sympatric conspecific *Elysia rufescens* contain secondary metabolites effective against certain types of cancer. The role of sacoglossan feeding history and sequestration of algal metabolites may influence the types and amounts of

anti-cancer chemicals produced. For example, Rao *et al.* (2008) reported the absence of kahalalide B, a chemical previously isolated in abundance from Hawaiian *E. rufescens*, from their sacoglossans despite sites and times identical with previous collections. While the authors suggest potential differences in microbial associates, another realistic explanation would be different algal hosts or past feeding histories.

Other sacoglossan species such as *Placida daguilarensis* have not been studied sufficiently since description for a full understanding of the diet breadth to be possible. Our recent record of the species in Japan, from Okinawa to Hokkaido (Hirano *et al.* 2006b, 2006c), was a considerable extension of the species known geographic range (namely Hong Kong). Working on a Sagami Bay population of *P. daguilarensis*, Hirano *et al.* (2007c) also expanded the known details of its algal associations from just *Bryopsis* and *Derbesia* to also *Pseudoderbesia* and *Pedobesia*. However, the species is most commonly associated with *Bryopsis* and prefers it to *Pedobesia*.

In contrast to these records of wider-than-expected diets, the degree of genus-level specificity for the *Codium* feeders in Okinawa was unexpected. Most *Codium*-feeding sacoglossans in other geographic regions will consume both *Codium* and *Bryopsis*; these algal genera are in different families but are structurally similar to a sacoglossan (thin-walled coenocytic siphons). With the Okinawan sacoglossan species, there appeared to be little to no trophic overlap between the two genera. For example, this study and one in Sagami Bay (Trowbridge *et al.* 2008a) supported earlier work by Jensen (2003) that *Elysia trisinuata* does not appear to feed on *Bryopsis*. The undescribed *Placida* sp. also appeared to feed just on *Codium*; we have no records of it on *Bryopsis*. In contrast, *Elysia setoensis* Hamatani, 1968 from Honshu does feed on both genera as well as several other genera (Trowbridge *et al.* 2008a). Furthermore, ecological work on *Placida* sp. (*sensu* Baba 1986) also reported the species fed on *Codium* and *Bryopsis* on Honshu shores with infrequent records from *Pedobesia* (Shimadu 2004, Shimadu *et al.* 2006, Kumagai 2009, Hirano, unpubl. data). Are these differences in degree of feeding specificity ecologically or genetically determined? Intriguingly, there was no evidence of monophagy in three sacoglossan species feeding on *Codium* in Sagami Bay, (Trowbridge *et al.* 2009b); they ate many of the approx. 20 species of *Codium* in Japan. Certainly, the sacoglossan species reported herein from Okinawa are reported on other *Codium* spp. elsewhere in their range.

Population abundances

The population abundances of the *Codium*-feeding sacoglossans (Figs. 5, 7) seasonally peak at an average of ca. 5 slugs per kg algae (for *Elysia trisinuata*), ca. 15 (two *Stiliger* spp.), and ca. 40 (undescribed *Placida* sp.). The largest of these species (*Elysia*) has the smallest population densities. How do

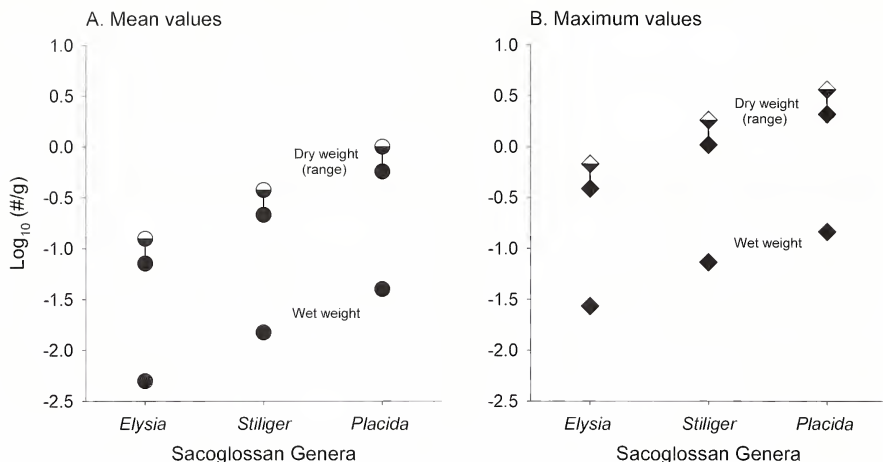


Figure 7. Comparison of population densities of sacoglossans based on the number per gram of algal wet weight or dry weight, using mean (A) or maximum (B) slug abundances. Because the wet to dry weight conversion was based on a published range (1 kg *Codium* wet weight would be ca. 40–70 g dry weight), a range of dry weight estimates is shown. The horizontal dotted lines indicate sacoglossan population density predicted for Okinawan latitude, based on the latitudinal trend of Atlantic species (Clark and DeFreese 1987).

these values compare to sacoglossan abundances reported for other regions? Clark and DeFreese (1987) reported 32.4 slugs/g dry weight of *Codium* in Connecticut (41°N) (for *Placida dendritica*) and 24.2/g dry weight of *Bryopsis* at 25°N (for *Placida kingstoni*). Based on sacoglossan populations spanning 40° latitude in the N Atlantic, Clark and DeFreese (1987) reported the following linear regression: $\log_{10}(\text{density}) = 0.1109(\text{latitude}) - 2.9683$. Based on our 23°N location at Okinawa, we would predict densities of ca. 0.4 specimens per g dry weight of algae (Fig. 7, note $\log_{10}$ scale).

Our study reported slug abundances based on algal wet weights, as a drying oven was not available. If the dry to wet weight ratio for *Codium fragile* (reported by Trowbridge 1998) can be extrapolated to *Codium geppiorum*, then 1 kg of *Codium* wet weight would be ca. 40–70 g dry weight. Consequently, our mean sacoglossan abundances would be estimated to be ca. 0.1 slugs/g dry weight algae (*Elysia*) to ca. 0.6–1.0 slugs/g (*Placida*) at 23°N, lower than predicted for Atlantic populations (Fig. 7A).

However, the Atlantic regression was based on peak densities rather than mean densities in the peak season. If we calculate peak densities based on maximum abundance of a species in a given collection, our estimates would be 0.4–0.7 (*Elysia* in July 2004) to 2.1–3.7 slugs/g dry weight (*Placida* in March 2006). Thus, the NW Pacific values would then be

comparable to or higher than those slug populations in the NW Atlantic Ocean (Fig. 7B). A more rigorous examination of Pacific sacoglossan species along a large latitude gradient would provide a more precise comparison. However, our preliminary comparison indicates that Pacific sacoglossan populations are not only highly diverse but also comparatively abundant for their latitude.

Furthermore, qualitative observations on websites or in guidebooks may contribute to misconceptions about species' abundance, diversity, and seasonality unless the sampling biases are explicitly indicated. Bolland repeatedly states on his website that many of the sacoglossans are extremely unusual to rare; yet our research group reliably finds these species during most of our surveys. For example, Bolland reported the undescribed *Placida* sp. (which he called *Placida dendritica*) as "very rare in the Keramas" and "currently unknown from the waters of Okinawa's main island" (<http://rbbolland.com/okislugs/placdend.html>) whereas we have found >320 specimens of this species on the main island. How do we explain these differences? The primary difference is probably one of methodology: we snorkeled in extremely shallow water where macroalgae proliferate, whereas Bolland and colleagues generally dive in deeper water, using scuba. Thus, the two research groups are investigating slightly different habitats. Furthermore, we have

taken a phytocentric approach, investigating large numbers of thalli of each potential algal host. Because Bolland's sampling methodology has not been specifically reported, we presume he and other colleagues (generally oriented more to nudibranchs than to sacoglossans) may have a less phytocentric approach. Consistent surveys by the same investigators at the same sites throughout many years will enhance the understanding of assemblages of sacoglossan opisthobranchs. Also, as well stated by Clark (1994: 901): "Once food species have been identified, sacoglossans can be relocated repeatedly". By reporting the algal associations in this paper, we hope other investigators can start reliably and quantitatively recording populations of subtropical sacoglossans. A comparable point was made by Bouchet *et al.* (2002: 427): "The existence of such species [very stenoeious] is revealed only if the hosts are appropriately sampled and scrutinized."

At special risk vs. predictably rare?

Different types of rarity

Are "sparse" or "uncommon" mollusc populations necessarily degraded or at "special risk" as Clark (1994), Bolland (web site), and others have suggested? Clark (1994) emphasized that one source of vulnerability of the Key West sacoglossans was the high incidence of direct development (and hence, low dispersal); this high incidence is unusual within the group. However, he also suggested that planktotrophic larvae may not necessarily disperse large distances, so all sacoglossans may be at "special risk" due to their host specificity and known habitat destruction or degradation.

Ecological theory, however, indicates that "uncommon" to "rare" species are not necessarily a conservation risk; ecologists have long realized that there are fundamentally different forms or types of "rarity" in species (Rabinowitz 1981). Species can be rare in different ways: by having a small geographic range, by having extreme habitat specificity, and by having small local populations (Table 5). The $2 \times 2 \times 2$ matrix

of the three traits lead to 8 cases: 7 of these denote different types of rare species and 1 indicates common species. If we characterize geographic range, habitat specificity, and local population abundance of sacoglossans, we would have a much better understanding of the vulnerability or conservation status of these species.

Larval development and population size

Most of the 100-150 recorded Japanese sacoglossan species have planktotrophic larvae (Trowbridge *et al.*, unpubl. data) and large geographic distributions and, thus, are probably not highly vulnerable to local disturbance or change. At least 8 of the 11 species discussed herein have planktotrophic larvae (*Caliphylla* sp., undescribed *Elysia* sp., and *Elysia rufescens* have not yet been investigated). Some sacoglossans (such as *Elysia setoensis* in Japan) do have a broad algal-host range with many genera of algae used; the broad host range can function as a buffer. Furthermore, temperate sacoglossan species often have high local population sizes (Clark and DeFreese 1987) and, hence, may often be considered ecologically common. *Elysia viridis* is an excellent example of this case with wide distribution, broad suite of algal hosts, and large population sizes (see Trowbridge *et al.* 2009c).

Sacoglossans that are more stenophagous in host associations, limited to one or just a few algal genera, could be considered (1) "predictably rare" (e.g., undescribed *Placida* sp. and *Elysia trisinuata*)—such that they can be found if the specific habitat is investigated—or (2) "sparse" (e.g., *Caliphylla* sp.)—such that the local population size is small and the species cannot be consistently located. The latter type of species is vulnerable to habitat loss such as the local or regional elimination of a host species (to disease, disturbance, pollution); the relative size of local populations and the capacity to disperse mediate the species' susceptibility to extrinsic factors. Yet, the situation discussed by Clark (1994) where specialists are "at special risk" of natural or anthropogenic change would occur primarily in species with restricted geographic distributions and/or limited dispersal due to direct development. Thus,

Table 5. Different forms of rarity based on three species traits: size of geographic range, habitat specificity, and local population size [based on Rabinowitz (1981)]. Of the eight possible combinations, seven cases denote different types of rarity and one case is the common species. NW Pacific sacoglossans generally fall in the two categories indicated with boldface, capitalized font.

Geographic distribution		Wide		Narrow	
Habitat specificity		Broad	Restricted	Broad	Restricted
Local population size	Somewhere large	Common	PREDICTABLE	Locally common	Endemic
	Everywhere small	Sparse	SPARSE	Sparse	Sparse

most NW Pacific sacoglossans are "predictably rare" to "sparse" rather than at "special risk" of local extinction.

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